

SUPPLEMENTATION OF VITAMIN E AND C IN FEED ON MEAT QUALITY, THIOBARBITURIC ACID REACTIVE SUBSTANCE (TBARS) AND MYOGLOBIN LEVEL OF MUSCOVY DUCK MEAT

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Received January 02, 2014; Accepted February 28, 2014

ABSTRAK

Penelitian ini bertujuan mengkaji suplementasi vitamin E dan C dalam pakan terhadap kualitas daging, kadar *thiobarbituric acid reactive substances* (TBARS) dan mioglobin daging itik Manila. Materi yang digunakan itik Manila jantan sebanyak 84 ekor dan perlakuan yang dicobakan ada 7 (tujuh) yang terdiri dari E₀C₀ : pakan tanpa disuplementasi vitamin E dan vitamin C, E₄₀₀ : pakan yang disuplementasi vitamin E sebesar 400 IU, E₆₀₀ : pakan yang disuplementasi vitamin E sebesar 600 IU, C₄₀₀ : pakan yang disuplementasi vitamin C sebesar 400 mg, C₆₀₀ : pakan yang disuplementasi vitamin C sebesar 600 mg, E₂₀₀C₂₀₀ : pakan yang disuplementasi vitamin E sebesar 200 IU dan vitamin C sebesar 200 mg, E₃₀₀C₃₀₀ : pakan yang disuplementasi vitamin E sebesar 300 IU dan vitamin C sebesar 300 mg, masing-masing perlakuan diulang sebanyak 4 kali. Rancangan percobaan yang digunakan Rancangan Acak Lengkap (RAL) dan masing-masing perlakuan diulang sebanyak 4 kali. Data dianalisis menggunakan analisis variansi. Hasil analisis ragam menunjukkan bahwa perlakuan berpengaruh sangat nyata ($P > 0,01$) terhadap kualitas fisik daging, kadar mioglobin dan angka TBARS. Simpulan penelitian ini adalah suplementasi vitamin E dan C mampu memperbaiki pH akhir daging itik Manila. Suplementasi vitamin E 300 IU dan C 300 mg pada pakan berprotein 21% dan energi metabolis 3100 kcal/kg mampu memperbaiki DIA, susut masak, flavor dan warna daging itik Manila, namun untuk menghasilkan keempukan yang tinggi sebaiknya pakan disuplementasi dengan vitamin E sebanyak 400 IU.

Kata kunci : mioglobin, senyawa volatil, kualitas daging

ABSTRACT

This research was aimed to analyze vitamin E and C supplementation in feed on meat quality, thiobarbituric acid reactive substances (TBARS) and myoglobin level of muscovy duck. This research used 84 Indonesian muscovy duck divided into 7 group of experimental diet, namely E₀C₀ : basal feed without vitamin E and C supplementation, E₄₀₀ : basal feed with 400 IU of vitamin E supplementation, E₆₀₀ : basal feed with 600 IU of vitamin E supplementation, C₄₀₀ : basal feed with 400mg of vitamin C supplementation, C₆₀₀ : basal feed with 600mg of vitamin C supplementation, E₂₀₀C₂₀₀ : basal with 200 IU of vitamin E and 200mg of vitamin C supplementation, E₃₀₀C₃₀₀ : basal feed with 300 IU of vitamin E and 300mg of vitamin C. A completely randomized design was applied and each treatment had 4 replications. The data were analyzed using analysis of variance. Analysis of variance showed that treatments significantly affect ($P > 0.01$) meat quality, myoglobin level and TBARS level. Vitamin E and C was proven able to improve final pH of muscovy duck meat. Supplementation of 300 IU of vitamin E and 300mg of vitamin C at feed with 21% of protein and 3100 kcal/kg of energy could improve DIA, cooking loss, flavor, and color of muscovy duck meat; however, the highest meat tenderness was resulted from 400 IU vitamin E supplementation.

Keywords: myoglobin, volatile compounds, meat quality

INTRODUCTION

Indonesian muscovy ducks, widely found in Java Island, are commonly under traditional breeding and unable to produce maximum performance and meat quality (CIVAS and FAO, 2006). Likewise, muscovy meat is less favorable in society due to its tough, dark, and musty meat.

Kim *et al.* (2008) found only 16% of white muscle fibers in duck breast meat but 100% of white muscle fibers in chicken breast meat. Besides, chicken breast has more protein than duck (22.0% vs 20.1%) but less fat than duck (1.8% vs 1.1%); accordingly, chicken pH is significantly lower than that of duck at 1-3 hour post mortem, but share common final pH at 24 hour. Qiao *et al.* (2001) reported that muscovy ducks, as well as other ducks, are mostly of red muscle fibers with minor white muscle fibers or 84% and 16%, respectively, which affects meat composition, biochemical and sensory characteristics.

Color still affects psychological response, economical value and consumer preferability toward food product. Meat color is determined by level and status of myoglobin pigment (Mancini and Hunt, 2005) and the appearing color depends on the order, age, sex (Wawro *et al.*, 2004), muscle type, feed, pre-slaughter treatment and stress, slaughtering method and storage condition (Haraf *et al.*, 2009). At 8 weeks old, myoglobin in red meat fowl is 0.4 mg/g, while in white meat fowl is 0.01 mg/g (Stadelman *et al.*, 1988). Myoglobin content increases along with the age. Myoglobin and hemoglobin in meat can speed up fat oxidation that causes pungency and off-flavor because the increase of myoglobin is followed by Fe. Iron Fe is catalyst to speed up oxidation rate and Fe level is affected by species, sex, age, muscle, myoglobin and hemoglobin activity and Fe (in ferrous), because Fe is easily oxidized and causes dark meat color (Meluzzi *et al.*, 2009; Apriyantono and Lingganingrum, 2001, Tang *et al.*, 2000; Barciela *et al.*, 2008; Min *et al.*, 2010; Yoon *et al.*, 2010).

Fat oxidation can be prevented or impeded by antioxidant whose usage to living fowl has to meet some requirements, among which are nontoxic, non-flavoring, non-coloring, effective in small amount, inexpensive, and readily available. Well-known antioxidant is vitamin C and vitamin E. Vitamin C has 2 hydroxyl groups are easily oxidized, so it will easily release electrons and hydrogen to donate to the free radicals so that free

radicals are not reactive or unstable (Sediaoetama, 1987) and as a regenerator of vitamin E (Rukmiarsih *et al.*, 2011). Vitamin E protects fatty acid from oxidation by trapping free radicals and is effective in cell membrane, while vitamin C is very efficient in trapping some compounds such as superoxide, hydrogen peroxide, hydroxyl radicals and peroxy radicals. There is synergic interaction between vitamin E and C, in which the former is lipophilic and the latter is hydrophilic, also vitamin C can degenerate radical-formed vitamin E. (Lavoisier, 1998; Lamid, 1995; Winarsi, 2011). The objective this study was to determine the effect of vitamin E and C supplementation in feed on meat quality, thiobarbituric acid reactive substances (TBARS) and myoglobin level of muscovy duck.

MATERIALS AND METHODS

The materials used were 84 nine-week-old male muscovy duck weighing 850-1100 g, kept in 28 litter cage compartments each containing three heads. Muscovy ducks were reared for five weeks and at 14 weeks old, two ducks were taken from each compartment and slaughtered to take the meat and to analyze its physical quality, thiobarbituric acid reactive substances (TBARS) level and myoglobin level.

Ducks were given basal feed containing 21% protein, 3100 kcal/kg feed metabolic energy (30% corn, 7% soy bean meal, 38.20% poultry meat meal, 6.10% oil, 0.10 % L-lysine HCl, 0.30 % DL-methionine, 0.20% topmix, 0.10% NaCl and 1% CaCO₃), powdered vitamin E (d- α -tokoferolasetat) and vitamin C (L-ascorbic acid). Ducks were New Castle Disease (NCD) vaccinated at first week. Drink water was supplied *ad libitum* and feed was given twice a day in the morning and afternoon.

Completely randomized design was applied, in which treatments were vitamin E and vitamin C supplementations to basal feed containing 21% protein and 3100 kcal/kg metabolic energy administered into seven groups, namely E₀C₀ : feed without Vit E and Vit C, E₄₀₀ : feed plus 400 IU vitamin E, E₂₀₀ : feed plus 600 IU vitamin E, C₄₀₀ : feed plus 400 mg/kg feed vitamin C, C₄₀₀ : feed plus 600 mg/kg feed vitamin C sebanyak, E₂₀₀C₂₀₀ : feed plus 200 IU vitamin E and 200 mg /kg feed vitamin C, and E₃₀₀C₃₀₀ : feed plus 300 IU vitamin E and 300 mg /kg feed vitamin C. Each treatment was subject to four replications. The obtained data were then analyzed using

analysis of variance and any differences were further subject to honestly significant difference Test.

The observed parameters were meat quality (pH, water holding capacity, cooking loss and meat tenderness), level of TBARS and myoglobin. Measurement was conducted using pH meter for pH (Bouton *et al.*, 1971), Hamm method for WHC (Soeparno, 2005) and Soeparno's (2005) method for cooking loss. TBARS level was estimated according to AOAC (1995). The 10g meat sample added with 50ml aquadest was mashed for two minutes, moved into distillation flask while being rinsed with 47.5 ml aquadest and added with 2.5ml HCL to reach 1.5 pH. Boiling stones were then folded into the mixture, attached to the distillator, distilled using high speed electric mantle heater for 10 minutes to obtain 50ml distillate. The distillate was stirred well, taken 5 ml then placed in reaction tube with cap then added with 5ml TBA reagent. Tube was closed, well shaken and heated in boiling water for 35 minutes. Blank solution: 5ml aquadest + 5ml TBA reagent was cooled for 10 minutes. Absorbance (D) was read using spectrophotometer, Z528nm with blank solution as zero point.

$$\text{TBA} = 7.2D \text{ (mg/100 kg sample)}$$

Myoglobin level according to Lerner (2009). From each sample 2 ml of the supernatant were saturated with 75 % ammonium sulphate (0.525 g.ml⁻¹) to precipitate the haemoglobin while keeping the mioglobin in the solution (1). Precipitated haemoglobin was separated by centrifugation at 2000 rpm at 21°C for 45 min. This solution was used for evaluation of mioglobin using the modified kinetic method with o-tolidine as described above. The results were processed statistically using software "Statgraphic Plus". The dependence of A630 on sample concentration was linear and the calculated relation was:

$$\text{mg.l}^{-1} = -0.0804722 + 14.6076 \cdot A630 \text{ (correlation coefficient; } r = 0.992784)$$

Research was conducted in muscovy duck experimental farm in Animal Science Faculty, Jenderal Soedirman University, Nutrition Laboratory PAU Gadjah Mada University, Laboratory of Food and Nutrition of Agricultural Technology Faculty Gadjah Mada University and Chemistry Laboratory of Mathematics and Science Program, Jenderal Soedirman University.

RESULTS AND DISCUSSION

Meat Quality

The pH of muscovy duck meat in this research was 5-5.49 which was relatively similar to 5.7 ± 0.05 of male muscovy by Wawro *et al.* (2004). Table 1 shows that E₀C₀ was significantly different ($P < 0.01$) from all other treatments; however, E₄₀₀, E₆₀₀, C₄₀₀, C₆₀₀, dan E₃₀₀C₃₀₀ were not different. This is because vitamin C and E function as antioxidant that prevents the forming of free radicals and increases oxidative stability of meat which affects muscle glycogen depletion rate. It was in line with Bou *et al.* (2006) that 150 mg/kg of α -tokoferol asetat supplementation in broiler ration for 32 days could increase chicken oxidative stability. El-habbak *et al.* (2011) reported that vitamin E and C have hydroxyl compound and are easy to donor electron and hydrogen to free radicals. Consequently, free radicals formation relatively more slowly and the cattle get healthier. In healthier cattle, muscle glycogen depletion rate is slower than that of stressed or sick animals. Accordingly, meat pH of muscovy E₀C₀ is lower than that of other treatments. It was in accordance with Choi *et al.* (2010) and Kim *et al.* (2009) that meat pH depletion rate was affected by antioxidant intake before slaughtering. Antioxidant effectively slow down the glycogen store depletion into lactic acid and prevent oxidative process by free radicals.

WHC of muscovy duck meat in this research ranged from 33.95 ± 0.09 to 36.52 ± 0.30 . This values were higher than that of Utami *et al.* (2011) namely 26.75 -33.77% and 26.13%, respectively. Table 1 shows that feed without vitamin E and C supplementation (E₀C₀) produced different WHC ($P > 0.01$) from all other treatments; E₄₀₀ was not different ($P > 0.05$) from C₄₀₀, C₆₀₀ and E₂₀₀C₂₀₀. Also E₂₀₀C₂₀₀ was not different from E₃₀₀C₃₀₀. It demonstrated that muscovy WHC was affected by level of vitamin E and C administered. Vitamin E effectively maintains cell membrane integrity and meat juice loss that eventually affects WHC (Petraci and Cavani, 2012), protects cell from endogenous free radicals (Rahman, 2003) and affects body fat (Rusmana *et al.*, 2008). Vitamin C functions in carnitine synthesis that essentially serves in fatty acid transport from cytosol to mitochondria in fat biosynthesis, therefore WHC in vitamin C treatment is lower than that of vitamin E. The lower intramuscular fat level, the lower is water holding capacity by meat protein.

Table 1. Meat Quality of 14 Weeks old Muscovy Duck Given Vitamin C and E Supplementation

Parameters	E ₀ C ₀	E ₄₀₀	E ₆₀₀	C ₄₀₀	C ₆₀₀	E ₂₀₀ C ₂₀₀	E ₃₀₀ C ₃₀₀
pH	5.00±0.00 ^a	5.49±0.15 ^b	5.41±0.06 ^b	5.38±0.09 ^b	5.29±0.17 ^b	5.39±0.10 ^b	5.42±0.01 ^b
WHC (%)	33.95±0.09 ^a	35.04±0.11 ^b	35.64±0.17 ^b	34.93±0.41 ^b	34.83±0.19 ^b	36.11±0.11 ^{bc}	36.52±0.30 ^c
Cooking loss (%)	36.60±0.65 ^d	36.13±0.48 ^d	33.47±1.14 ^c	32.43±0.80 ^{bc}	33.04±0.66 ^{bc}	31.55±0.57 ^b	27.48±0.74 ^a
Tenderness (kg/cm ²)	5.80±0.37 ^b	4.76±0.39 ^a	5.38±0.27 ^{ab}	5.80±0.37 ^b	6.85±0.53 ^c	6.13±0.44 ^{bc}	5.94±0.40 ^b

E₀C₀: feed without Vit E and Vit C supplementation; E₄₀₀: feed plus 400 IU of vitamin E; E₆₀₀: feed plus 600 IU of vitamin E; C₄₀₀: feed plus 400 mg/kg feed of vitamin C; C₆₀₀: feed plus 600 mg/kg feed of vitamin C; E₂₀₀C₂₀₀: feed plus 200 IU vitamin E and 200 mg /kg feed of vitamin C and E₃₀₀C₃₀₀: feed plus 300 IU of vitamin E and 300 mg /kg feed of vitamin C. ^{abcd} shows highly significant (P<0.01)

Cooking loss of muscovy meat in this research ranged from 27.48 ± 0.74 to 36.60 ± 0.65%. These values were lower than that of Utami *et al.* (2011) but higher than that of Omojola (2007) on male muscovy namely 40.18% and 25.50%, respectively. E₀C₀ was not different (P>0.05) from E₄₀₀, and E₆₀₀ was not different from C₄₀₀ and C₆₀₀. Furthermore, E₂₀₀C₂₀₀ was not different from C₄₀₀ and C₆₀₀, but E₂₀₀C₂₀₀ was different from E₃₀₀C₃₀₀ (Table 1). Cooking loss process deals with water holding capacity as informed by Soeparno (2005) that the higher water holding capacity, the less juice loss during cooking, besides cooking loss is also affected by meat intramuscular fat. Muscle with high intramuscular fat tends to have high WHC and low cooking loss, because intramuscular fat resists or lessens meat juice extracted during cooking. Prawirokusumo (1990) stated that vitamin E is fat-soluble, effectively prevents fat oxidation that damages tissues; therefore, cooking loss when boiling meat can be reduced. Linder (1992) supported that at molecular level, vitamin C becomes reductive like vitamin E and active form. This characteristic is assumed to be able to defend cell stability from damage so that meat juice loss can be resisted.

Meat tenderness of 14 weeks old male muscovy that supplemented with vitamin C and E in feed have significantly effect (P<0.01). E₀C₀ was different from (P<0.01) all treatments. E₄₀₀ was not different from (P>0.05) E₆₀₀, but E₆₀₀ was not different from (P>0.05) C₄₀₀, E₂₀₀C₂₀₀ and E₃₀₀C₃₀₀. Vitamin E is fat-soluble that maintains

plasma membrane integrity (Khan *et al.*, 2011) and eventually lowers meat juice loss so that drip depletes and WHC increases. This was in accordance with Li *et al.* (2009) that vitamin E supplementation in feed decrease the drip loss and increase tenderness but not significantly affected carcass production, while vitamin C is water-soluble antioxidant that significantly serves in forming intercellular collagen and fat metabolism. Collagen is a type of protein as the main component in connective tissue (Soeparno, 2005), so vitamin C efficacy in tenderness is still unstable. On the other hand, vitamin C synergized vitamin E will effectively increase meat tenderness.

TBARS and Myoglobin Level

The effect of vitamin E and C supplementations in feed resulted in 0.43 – 0.86 mg/100 g of TBARS level, or higher than that of muscovy fed with *Pluchea indica* scoring 0.302 - 0.359 mg/100 g (Rukmiasih *et al.*, 2009). Table 2 showed that feed supplemented with vitamin C and E significantly affected (P<0.01) myoglobin of 14 weeks old male muscovy. E₂₀₀C₂₀₀ was not different (P>0.05) from E₃₀₀C₃₀₀, also between E₀C₀ and E₄₀₀ and between E₄₀₀ and C₄₀₀, C₆₀₀. Oxidation process gets faster when in alkali surrounding. Free oxygen in the air will oxidized double chained unsaturated fatty acid in food, and fatty acid oxidation would be followed by H₂O₂ formation that induced pungency. However, if antioxidant exists in feed, the active peroxide formed would react with antioxidant to prevent

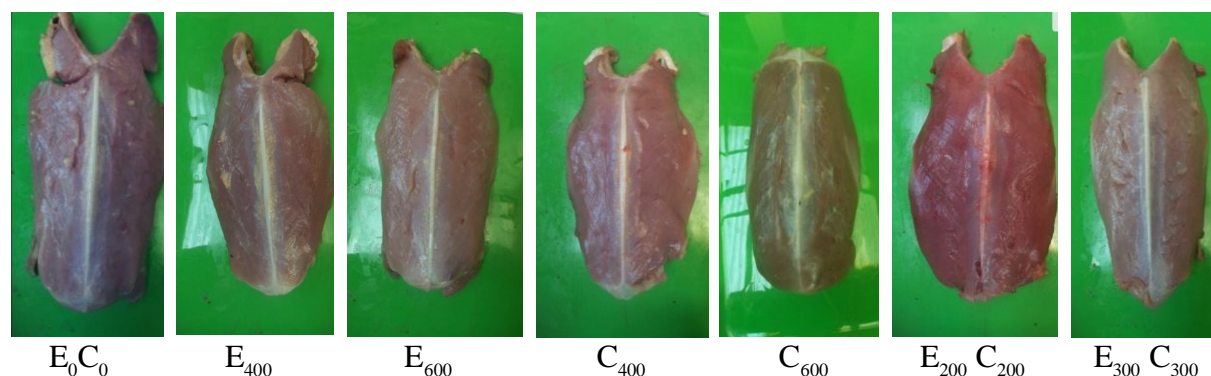
free radicals formation and eventually slow down the formation of malonaldehyd (Lukman *et al.*, 2007). Table 2 shows that antioxidant intake either vitamin C or E could impede oxidation and malonaldehyd formation in muscovy meat, those were observed from lower TBARS value compared to muscovy fed without vitamin C and E supplementation. Oxidative rate would differ due to the effect of characteristics and efficacy of antioxidant intake, meat fatty acid and unsaturated fatty acid. Fat level and unsaturated fatty acid of muscovy skinned meat in each treatment was relatively high, in that E_0C_0 was 6.72% and 42.64%, E_{400} was 556% and 49.55%, E_{600} was 7.23% and 37.64%, C_{400} was 7.12% and 46.85%, C_{600} was 6.89% and 43.41%, $E_{200}C_{200}$ was 7.07% and 32.86%, $E_{300}C_{300}$ was 7.11% and 45.01%. Furthermore, peroxide and TBARS scores according to Gheisari (2011) was positively correlated. Critical control point of meat tested by TBARS was 15 mg/kg sampel (Sanger,2010).

Myoglobin is monomeric protein serves as oxygen storage site on skeletal muscle cell (striated muscle). This protein is composed of at least 154 amino acid chains and one heme compound (porphyrin chain with one Fe atom). Myoglobin level of breast meat of muscovy ranged from 4.98 ± 1.61 to 9.07 ± 0.57 mg/g. Myoglobin level in this research was higher than that of Gheisari (2011) stated that chicken myoglobin was 0.31 ± 0.07 mg/g. Feed supplemented with vitamin C and E significantly affected ($P < 0.01$) myoglobin of 14 weeks old male muscovy. $E_{300}C_{300}$ was not different from E_0C_0 , E_{400} , E_{600} , C_{400} , C_{600} and $E_{200}C_{200}$. C_{400} and C_{600} was different from E_0C_0 , E_{400} and E_{600} . $E_{200}C_{200}$ was different from E_0C_0 (Table 2 and Figure 1). The lowest muscovy myoglobin level was due to feed supplemented with vitamin C, because vitamin C can increase triglyceride and affect muscle glycogen stores. Glycolysis rate became slower causing denaturation of muscle

Table 2. Myoglobin and TBARS Level of 14 Week old Muscovy Duck Meat Given Vitamin C and E Supplementation

Parameters	E_0C_0	E_{400}	E_{600}	C_{400}	C_{600}	$E_{200}C_{200}$	$E_{300}C_{300}$
TBARS (mg MA/100 g)	0.86 ± 0.14^c	0.82 ± 0.01^c	0.52 ± 0.00^{ab}	0.52 ± 0.01^{ab}	0.62 ± 0.01^b	0.44 ± 0.01^a	0.43 ± 0.01^a
Myoglobin level (mg/g)	9.07 ± 0.57^b	8.62 ± 0.98^b	8.09 ± 1.34^b	5.16 ± 2.17^a	4.98 ± 1.61^a	5.74 ± 1.34^a	7.41 ± 0.13^{ab}

E_0C_0 : feed without Vit E and Vit C supplementation; E_{400} : feed plus 400 IU of vitamin E; E_{600} : feed plus 600 IU of vitamin E, C_{400} : feed plus 400 mg/kg feed of vitamin C; C_{600} : feed plus 600 mg/kg feed of vitamin C, $E_{200}C_{200}$: feed plus 200 IU vitamin E and 200 mg /kg feed of vitamin C and $E_{300}C_{300}$: feed plus 300 IU of vitamin E and 300 mg /kg feed of vitamin C. ^{abcd} shows highly significant ($P < 0.01$)



Figures 1. Meat Color of Each Treatment

protein including myoglobin. Nelson and Cox (2008) stated that myoglobin has high oxygen holding capacity, making it easily oxidized, and turning into bright red (Mb) oxidation. Fletcher (2003) and Allen (2009) stated that myoglobin consists of heme and globin compound. Heme compound is hydrophobic consisting of one Fe ion with four nitrogen porphyrin rings. The sixth Fe bond is easily oxidized that turns meat color into dark. Zouari *et al.* (2010) mentioned that vitamin E prevents lipid oxidation, is effective to stabilize meat color, but excessive amount will lose the red color in meat because vitamin E can induce metmyoglobin very fast. Besides myoglobin, meat color is also affected by the extracted hemoglobin when slaughtered. Hemoglobin level of each treatment at slaughtering showed that E_0C_0 was 11.28 ± 2.88 g/dl; E_{400} was 14.75 ± 3.76 g/dl; E_{600} was 12.55 ± 3.76 g/dl; C_{400} was 16.35 ± 2.06 g/dl; C_{600} was 11.70 ± 1.57 g/dl; $E_{200}C_{200}$ was 13.40 ± 2.51 g/dl and $E_{300}C_{300}$ was 13.18 ± 2.01 g/dl.

CONCLUSION

Supplementation of 300 IU vitamin E and 300 mg vitamin C into feed with 21% protein and 3100 kcal/mg metabolic energy can recover WHC, cooking loss, flavor and meat color of muscovy duck, however, the highest meat tenderness was resulted from 400 IU vitamin E supplementation.

ACKNOWLEDGMENTS

The author would like to thank to Directorate General of Higher Education, Ministry of Education and Culture for the Doctorate scholarship in Post Graduate Animal Science Study Program, Gadjah Mada University Yogyakarta. Gratitude also goes to the Dean of Animal Science Faculty, Jenderal Soedirman University for granting study permit.

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